

Figure 1: Novel Gene Sequence Analysis

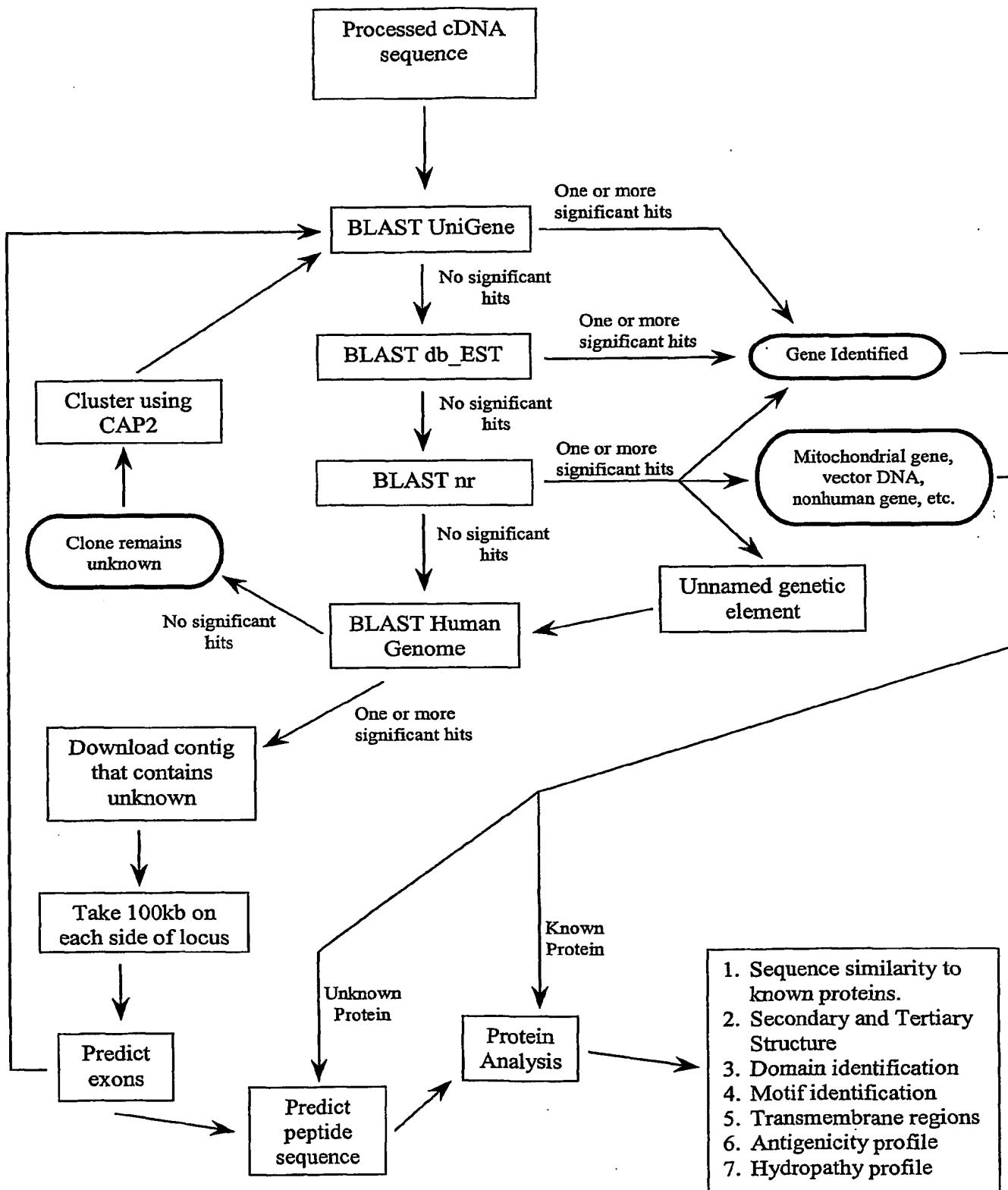


Figure 2: Primer efficiency testing. A standard curve of Ct versus log of the starting RNA amount is shown for 2 genes.

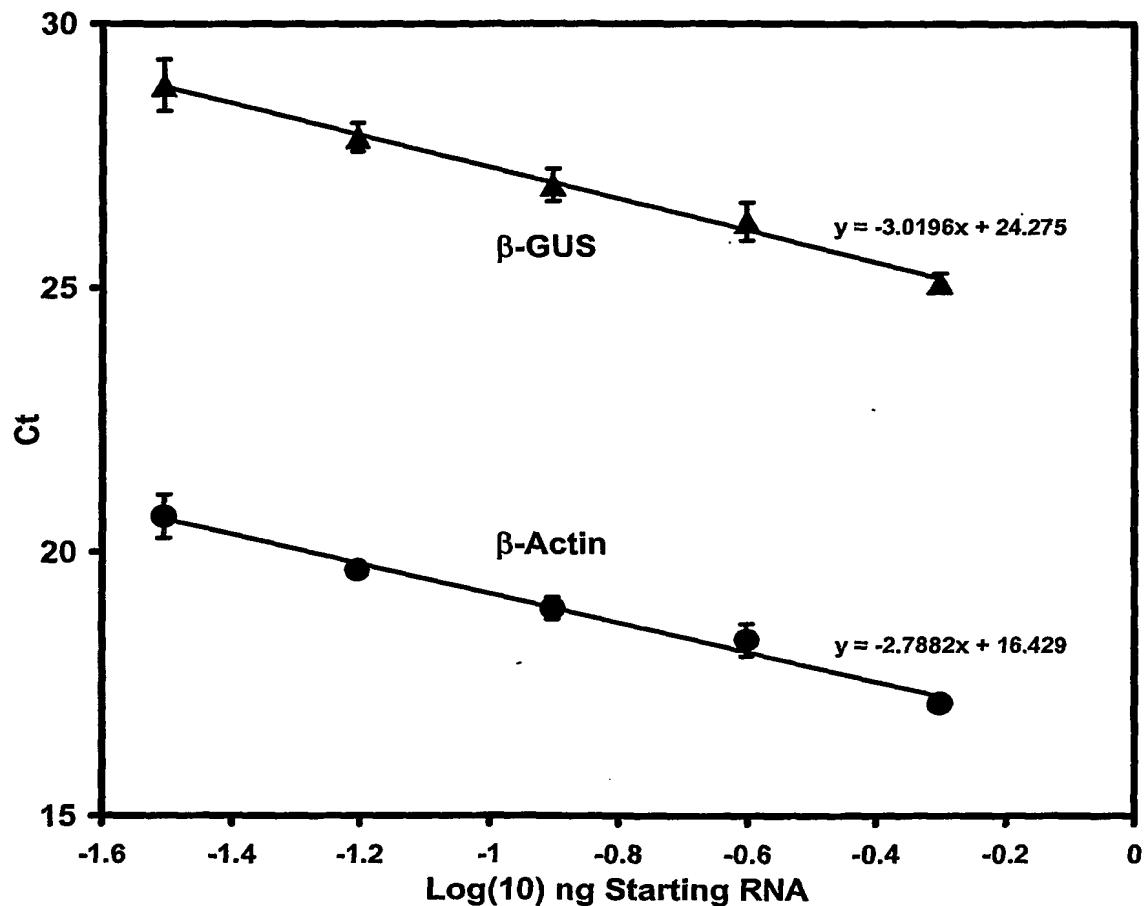


Figure 3: Kits for discovery of, or application of diagnostic gene sets**A. Contents of kit for discovery of diagnostic gene sets using microarrays**

1. Sterile, endotoxin and RNase free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
- 3.-PBS (phosphate buffer saline; needed when method of example 8 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)
 - For fluorescence microarray expression profiling:
 - Reverse transcriptase and 10x RT buffer
 - T7(dT)24 primer (primer with T7 promoter at 5' end)
 - DTT
 - Deoxynucleotides 100mM each
 - RNase inhibitor
 - 2nd strand cDNA buffer
 - DNA polymerase
 - Rnase H
 - T7 RNA polymerase
 - Ribonucleotides
 - In Vitro transcription buffer
 - Cy3 and Cy5 labeled ribonucleotides
7. Microarrays containing candidate gene libraries
8. Cover slips for slides
9. Hybridization chambers
10. Software package for identification of diagnostic gene set from data
 - Contains statistical methods.
 - Allows alteration in desired sensitivity and specificity of gene set.
 - Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
12. Kit User Manual

B. Contents of kit for application of diagnostic gene sets using microarrays

1. Sterile, endotoxin and RNase free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
- 3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence microarray expression profiling:

- Reverse transcriptase and 10x RT buffer
 T7(dT)24 primer (primer with T7 promoter at 5' end)
 DTT
 Deoxynucleotides 100mM each
 RNase inhibitor
 2nd strand cDNA buffer
 DNA polymerase
 Rnase H
 T7 RNA polymerase
 Ribonucleotides
 In Vitro transcription buffer
 Cy3 and Cy5 labeled ribonucleotides
7. Microarrays containing candidate gene libraries
 8. Cover slips for slides
 9. Hybridization chambers
 10. Software package for identification of diagnostic gene set from data
 Contains statistical methods.
 Allows alteration in desired sensitivity and specificity of gene set.
 Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
 12. Kit User Manual

C. Contents of kit for application of diagnostic gene sets using Real-time RT-PCR

1. Sterile, endotoxin and RNase free blood collection tubes
 2. Alcohol swabs, tourniquet, blood collection set
 3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
 4. Cell lysis buffer
 5. RNA isolation kit
 6. Substrates for real time RT-PCR (may vary for various real-time PCR techniques:
 poly dT primers, random hexamer primers
 Reverse Transcriptase and RT buffer
 DTT
 Deoxynucleotides 100 mM
 RNase H
 primer pairs for diagnostic and control gene set
 10x PCR reaction buffer
 Taq DNA polymerase
 Fluorescent probes for diagnostic and control gene set
 (alternatively, fluorescent dye that binds to only double stranded DNA)
 reaction tubes with or without barcode for sample tracking
 96-well plates with barcode for sample identification, one barcode for entire set, or individual barcode per reaction tube in plate
7. Software package for identification of diagnostic gene set from data
 Contains statistical methods.
 Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database server

8. Password and account number to access central database server.
9. Kit User Manual

FIGURE 4

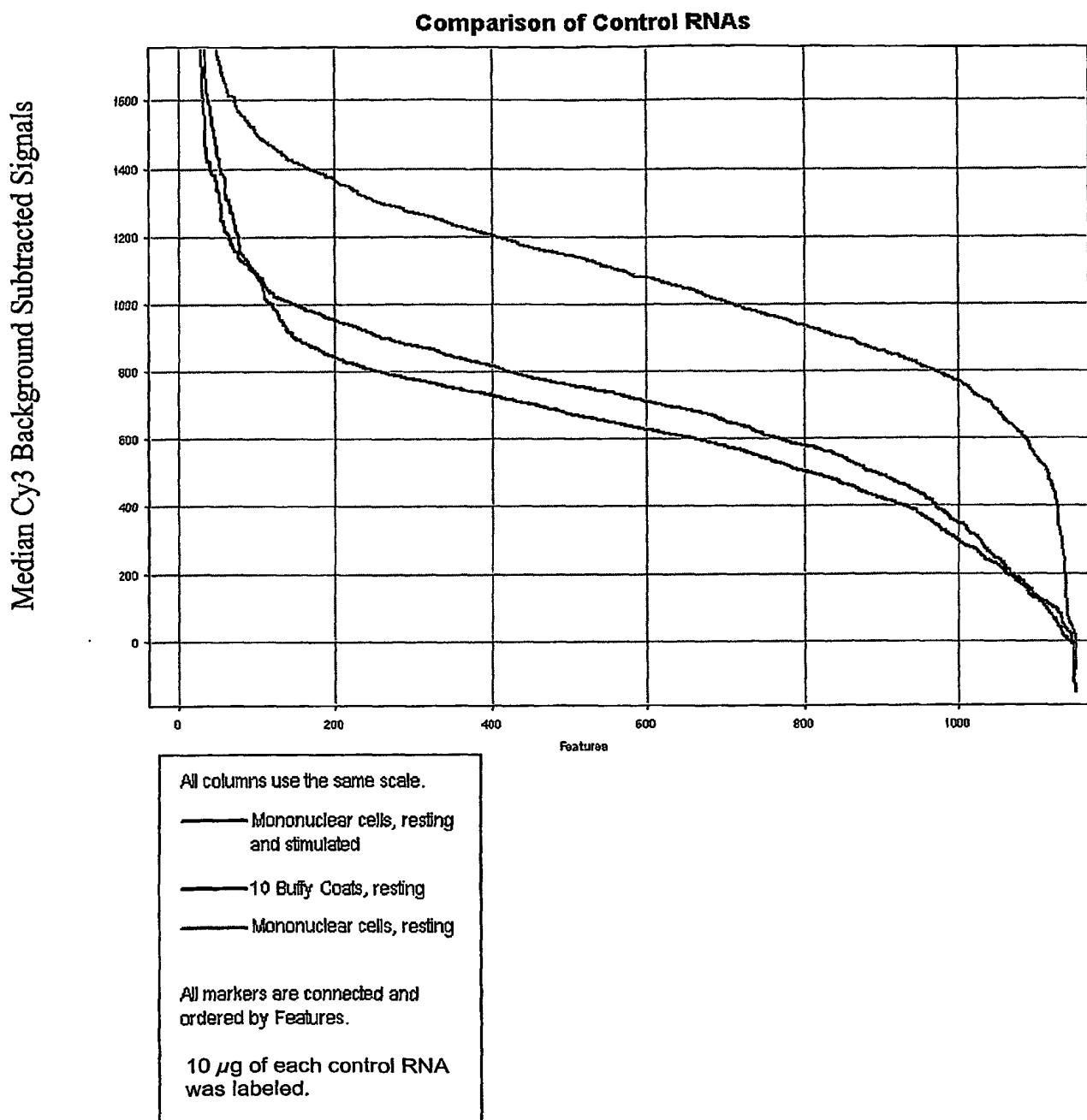
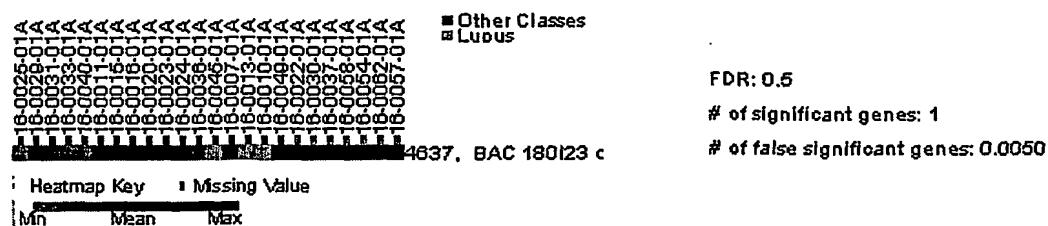


Figure 5: SLE diagnostic genes and algorithms**A.****B.**

Lupus	Control		
Sample	Ratio	Sample	Ratio
16-0022-01	1.05	16-0025-01	0.60
16-0030-01	0.96	16-0029-01	0.75
16-0037-01	0.87	16-0031-01	0.63
16-0058-01	1.05	16-0033-01	0.62
16-0054-01	0.99	16-0040-01	0.61
16-0062-01	0.98	16-0015-01	0.72
16-0057-01	1.14	16-0016-01	0.78
		16-0020-01	0.79
		16-0023-01	0.71
		16-0024-01	0.69
		16-0036-01	0.65
		16-0045-01	0.59
		16-0007-01	0.77
		16-0013-01	0.60
		16-0010-01	0.57
		16-0049-01	0.75

Lupus	Control
Average Ratio	1.00
Std Dev of Ratio	0.08
Fold Change	1.48

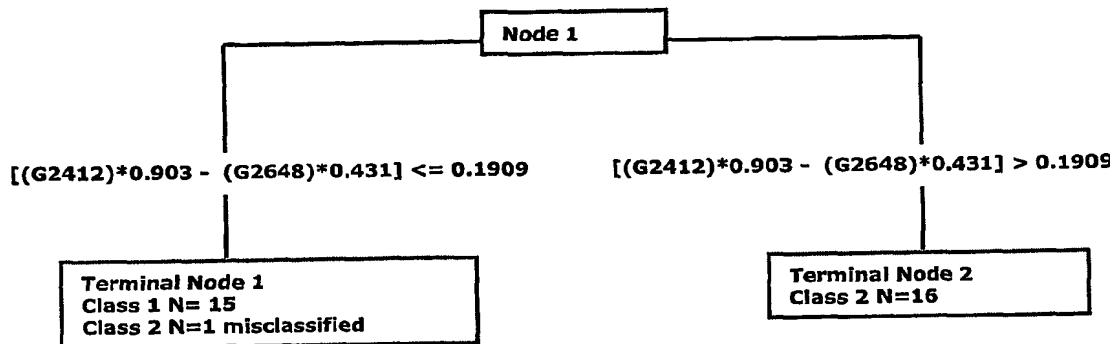
C.

Model #	Relative genes Cost	SEQ ID 50mer	Locus	Nominal Description	CART Splitter	CART Value for Dx SLE
Model I 2	0.118	514	NM_002946	replication protein A2 (32kD)	co-1st	$[(2412)*0.903 - (2648)*0.431] \leq 0.1909$
		510	NM_004510	interferon-induced protein 75	co-1st	$[(2412)*0.903 - (2648)*0.431] \leq 0.1909$
Model I 3	0.125	514	NM_002946	replication protein A2 (32kD)	co-1st	$[(2412)*0.903 - (2648)*0.431] \leq 0.1909$
		510	NM_004510	interferon-induced protein 75	co-1st	$[(2412)*0.903 - (2648)*0.431] \leq 0.1909$
		509	BC002409	actin, beta (ACTB)	2nd	$(G1436) > 0.0868$
Model II 1	0.612	504	W16552	PKR	1st	$(5067) > 0.1030$
Model II 3	0.686	504	W16552	PKR	1st	$(5067) > 0.1030$
		875	AK024756	hypothetical protein FLJ21103	2nd	$(G1025) \leq 0.3968$
		876	AK024969	hypothetical protein DKFZp566I133	3rd	$(G1035) \leq 0.0073$
Model II 5	0.745	504	W16552	PKR	1st	$(5067) > 0.1030$
		874	AK024240	cDNA FLJ14178 fts	2nd	$(G1003) > 0.2105$
		875	AK024756	hypothetical protein FLJ21103	2nd	$(G1025) \leq 0.3968$
		873	AK024202	heat shock 90kD protein 1, alpha	3rd	$(G1001) \leq -0.3107$
		876	AK024969	hypothetical protein DKFZp566I133	3rd	$(G1035) \leq 0.0073$

D.

	Model	Sensitivity	Specificity	Relative Cost
Training Set	Model 1 (2 genes)	100	94	
	Model 1 (3 genes)	100	100	
10-fold Cross Validation	Model 1 (2 genes)	100	88	0.118
	Model 1 (3 genes)	93	94	0.125

E.



F.

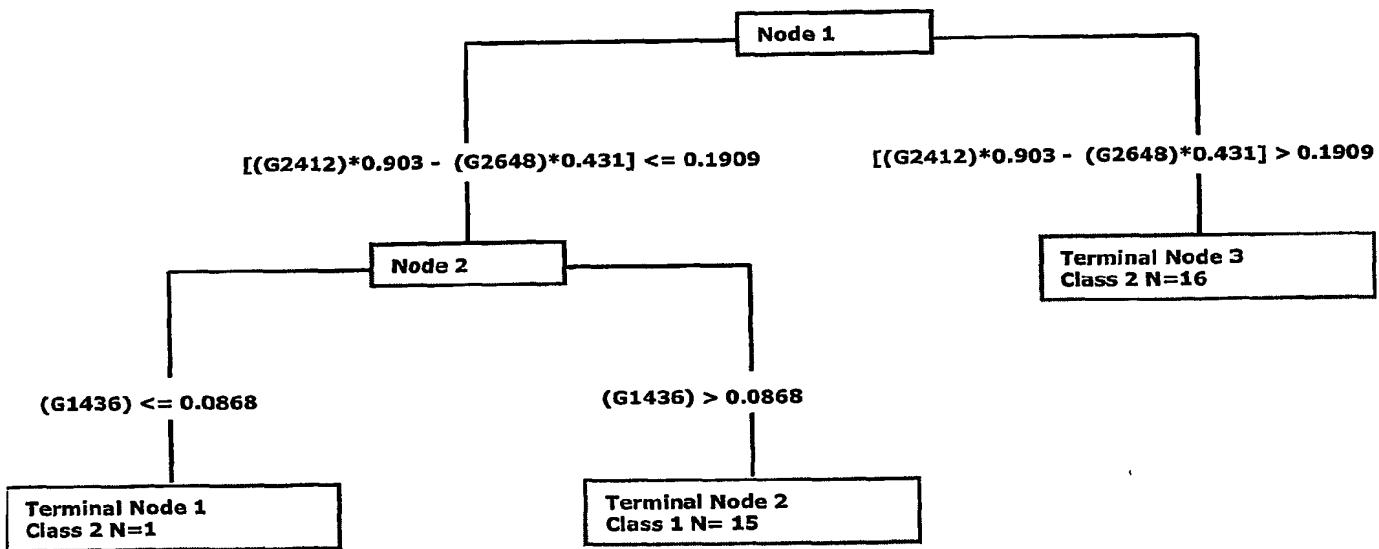


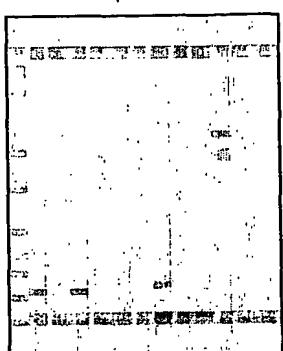
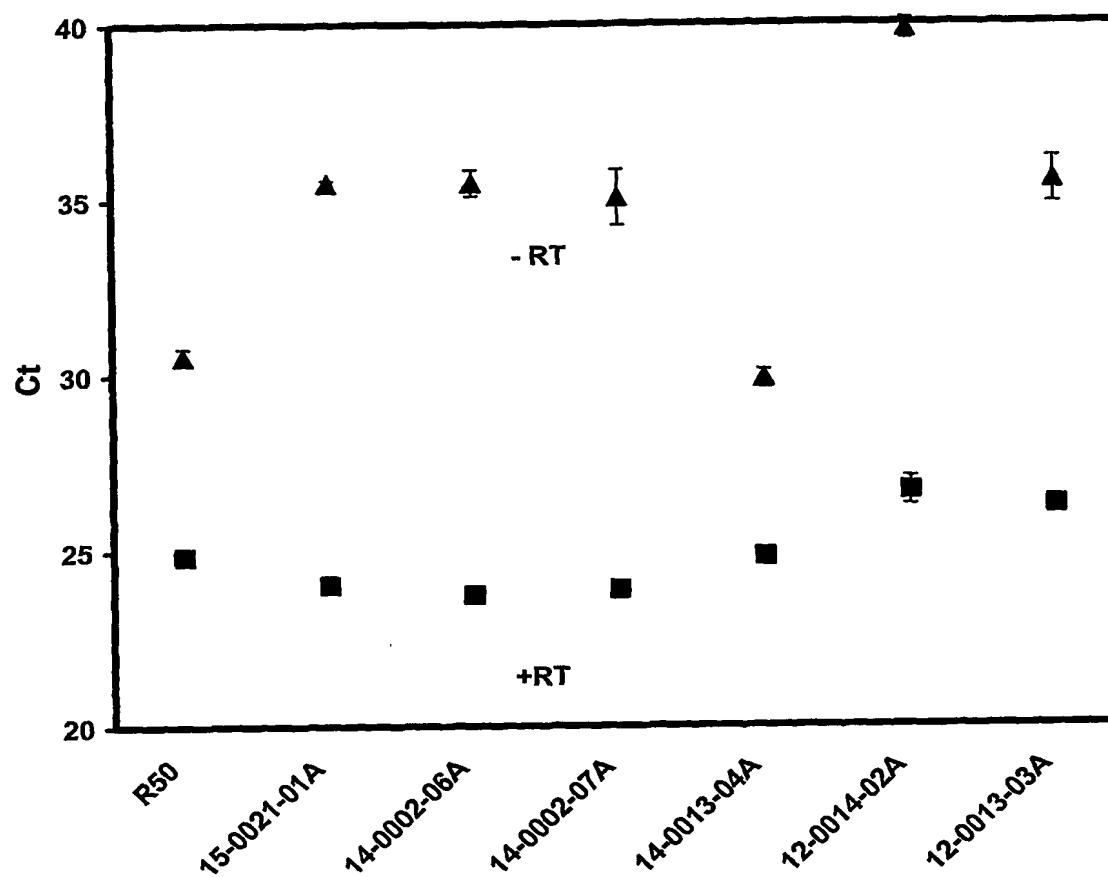
Figure 6. Endpoint testing of PCR primers**A.**

Figure 7: Validation of differential expression of Granzyme B in CMV patients using Real-time PCR

A.



B.

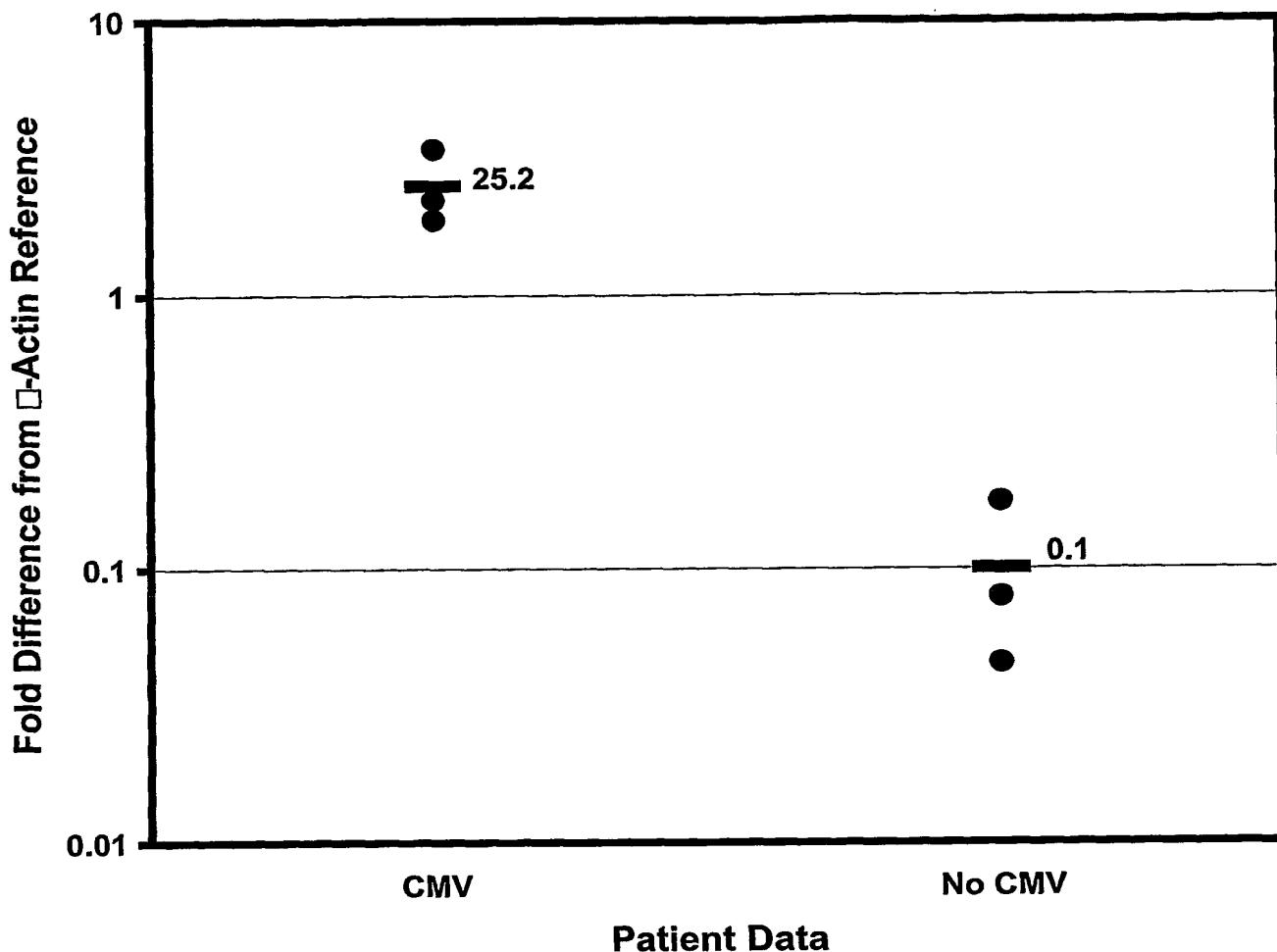
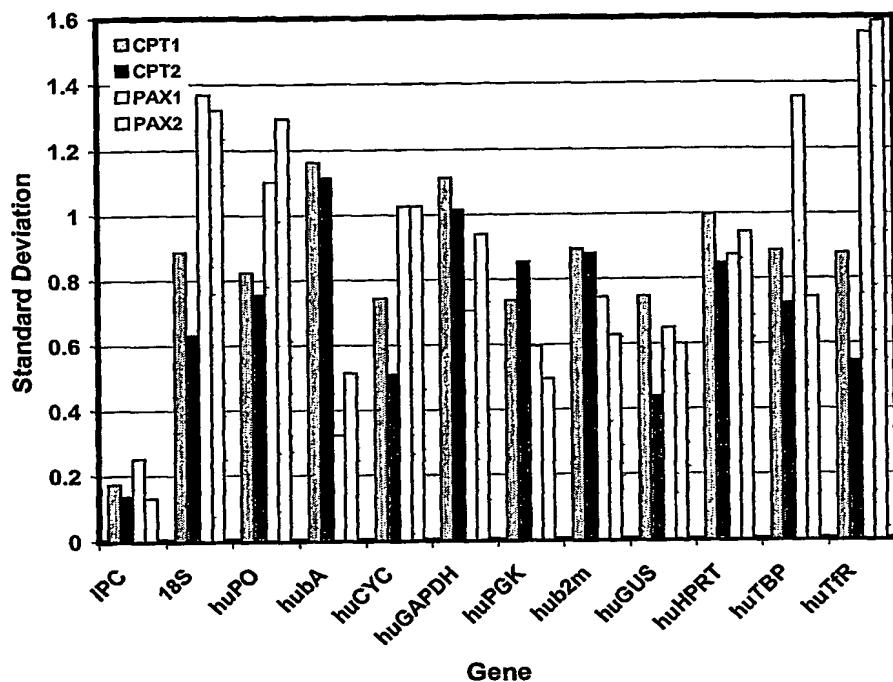
QPCR of Granzyme B

Figure 8**Variation of Control Genes from PAX RNA
(2ug) and CPT RNA (0.5 ug)****Intensity of Control Genes from PAX RNA
(2ug) and CPT RNA (0.5 ug)**